RAPD analysis on genomic DNA of male and female plants of *Acer negundo* L.

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Abstract Genomic DNAs were isolated from both male and female plants of *Acer negundo* L., by modified CTAB method of Doyle and Doyle for plant genomic DNA isolation and consequently tested with RAPD technique. Some sex-related specific oxyribonucleic acid bands were amplified by a group of decamer oligonucleotide random primers. This indicated that some genetic marks related to sexes of the species were found, which laid a foundation for sapling sex identification of *Acer negundo* L.

Key words: Genomic DNA, RAPD, Sex identification

Introduction

RAPD is a widely adopted molecular biological technique in many fields including forestry. J. I. Hormaza *et al.* (1994) applied this technique to sex-related research on dioecious tree species *Pistacia vera* L. and resulted very well. A random primer that produces specific amplification solely for female plant was screened. It was supposed the specific amplification was closely linked to female sex control gene. And the random primer was proved applicable to seedling sex identification of *Pistacia vera* L. by systematical experiments. A preliminary study on *Acer negundo* L., a dioecious tree species, was carried out with RAPD technique and some sex-related genetic marks were found out.

Material and method

Experiment material

Bulked young leaves of male or female trees of *Acer negundo* L., were collected, each sample contained leaves from 5 plants of the same sex.

Isolation of genomic DNA

Refer to modified CTAB method of Doyle and Doyle for genomic DNA isolation[1].

Purity and concentration determination of genomic DNA

OD value was determined by spectrophotometer. The most important OD value for DNA purity test is the ratio of 260 nm/280 nm. Most of DNA solutions got through the above method valued about 1.8-1.9.

The OD values and concentrations of genomic DNA solutions used for RAPD analysis in the paper from male and female plants of *Acer negundo* L. were as follows:

Table 1. The quality indexes of tested genomic DNA solutions

Sex	OD260	OD260/280	DNA Conc./mg•mL^{-1}
Female	0.1245	1.8246	1.245
Male	0.1308	1.8370	1.308

It is commented to be a better way for genomic DNA quality check. Took 5-10 uL DNA solution, added 10 uL of loading buffer (0.25% bromophenol blue; 40% sucrose (w/v)), sampled in the wells of 0.8% agarose gel. Meanwhile standard DNA marker was sampled. The electrophoresis was done in 0.5 TBE electrode buffer for about 1h. The gel was then dyed in 0.5 ug/mL EB for 30 min, and checked under UV light, if the band from the tested genomic DNA was clean without RNA contamination, and its molecular weight was around 23 kb, the DNA solution was good enough for RAPD analysis.

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Fig. 1. Genomic DNA from young leaves of female and male plants of *Acer negundo* L. were tested by agarose gel electrophoresis

The solutions of newly extracted genomic DNA were diluted with TE buffer in gradient, and the differently diluted solutions were sampled separately in wells of 0.8% agarose gel. The most slightly visible band under UV light indicated correspondingly the optimal concentration of genomic DNA for RAPD test.

PCR reaction

4 groups (A, B, C, D) of decamer oligonucleotide random primers produced by American Operon Com. were utilized. The total volume for amplification was 25 ul, including 2.5 ul DNA polymorphic reaction buffer (100m mol/L Tris- HCl (pH 8.0), 500 m mol/L KCl, 20 m mol/L MgCl); 2.0 ul dNTPs; 1.5 ul primer; 1.5 ul (10 ng) genomic DNA; 2.5 ul Taq DNA polymorase (1.25 u), and ddH_2O in supplement.

RAPD reaction program was as follows: preparatory denatured for 120 s at 94℃; denatured for 60 s at 94℃; replication for 120 s at 36℃; extension for 120 s at 72℃, together 45 cycles included, and finally kept the temperature at 72℃ for 300 s.

Amplified products were tested by 1.2% agarose gel electrophoresis, Lambda DNA/EcoR I or Hind III was sampled simultaneously as control. The gel was dyed in 0.02% EB solution, and examined (photographed) under UV light.

Result analysis

Primer A13 amplified respectively 4 oxyribonucleic acid bands from genomic DNA of female plant, whereas 6 bands from genomic DNA of the male, including a remarkably bright band with about 300 bp (Track 1, 2 from the left in Fig. 2).

Genomic DNA of male plant had 2 more bands between 600-700 bp of molecular weight than the female, amplified by A20 (Track 3, 4 from the left in Fig. 3).

Genomic DNA of female plant was amplified 5 more bands between 300-600 bp than male plant by primer B04 (Track 1, 2 from the left in Fig. 4).

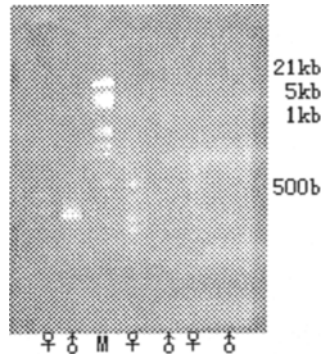

Fig. 2. RAPD test result by primer A13

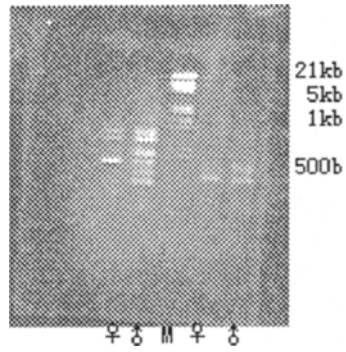

Fig. 3. RAPD test result by primer A20

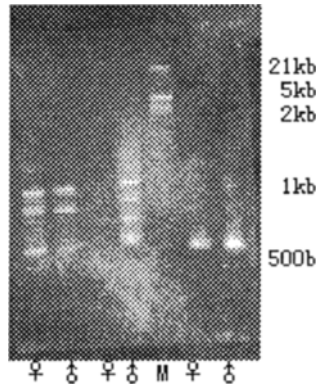

Fig. 4. RAPD test result by primer B04

Amplified by double primers A07+A08, genomic DNA of male plant had 3 bands, but the female had only 1 band (Fig. 5). The difference was distinct.

Fig. 5. RAPD test result by primers A07&A08

Amplified by double primers A19+A20, female plant had 4 bands, while the male had only 2 bands (Track 6, 7 from the left in Fig. 3).

Amplified by double primers of B04+B05, genomic DNA of male plant had 3 bands, but the female just had 2 bands (Track 6, 7 in Fig. 4).

Conclusion

Some specific oxyribonucleic acid bands were determined with genomic DNA either from male or female plants of *Acer negundo* L., indicating some sex-related genetic marks of the species were found. This work laid a foundation for sapling sex identification of *Acer negundo* L. in the future.

Compared with many other methods for plant genomic DNA isolation, the modified CTAB method of Doyle and Doyle was the most efficient but simple one, it is recommendable.

Application of double primers to RAPD test has been proved to be beneficial. Theoretically speaking, double primers may gain more information than normally single one in RAPD test, there will be $N \times (N-1)/2$ combinations out of N primers, that offers more chances for a single primer to be utilized. More tests can be done with certain number of primers.

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